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# ANTICANCER AND CELL CYCLE ANALYSIS OF PLANT EXTRACT OF MOMORDICA CHARANTIA ON BREAST ADENOCARCINOMA CANCER CELL LINES

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## ABSTRACT
Bitter melon (*Momordica charantia*) is an herbal plant grown in traditionally known for its medicinal properties such as antidiabetic, antitumorous, anticancer, anti-inflammatory, antiviral, and cholesterol lowering effects. The main constituents of bitter melon are responsible for various therapeutic effects such as triterpene, protein, steroid, alkaloid, inorganic, lipid, and phenolic compounds. The protein constitutes in bitter melon including protein MAP-30, alpha–momorcharin, and beta–momorcharin were shown to have the ability for fighting against HIV syndrome ect. A steroid, charantin contain mainly in the aerial parts, has been proven for its antidiabetic activity. The area of diet and cancer prevention research is a dynamic one, producing new theories and findings the majority of research on diet and cancer suggests that eating fruits, vegetables, whole grains and beans will lower the risk of developing cancer. This review focuses on foods that will help in lowering the risk of cancer.

**KEYWORDS:** Phytoconstituents, MAP-30, anticancer agents.

## INTRODUCTION
Bitter melon contains an array of biologically active plant chemicals including triterpenes, proteins, and steroids. One chemical has clinically demonstrated the ability to inhibit the enzyme guanylate cyclase that is thought to be linked to the cause of psoriasis and also necessary for the growth of leukemia and cancer cells. In addition, a protein found in bitter melon, momordin, has clinically demonstrated anticancer us activity against Hodgkin's lymphoma in animals. Other proteins in the plant, alpha- and beta-momorcharin and cucurbitacin B, have been tested for possible anticancerous effects. (Cunnicket al., 1993). A chemical analog of these bitter melon proteins has been developed, patented, and named "MAP-30"; its developers reported that it was able to inhibit prostate tumor growth. Another clinical study showed that MAP-30's antiviral activity was also relative to the herpes virus in vitro.(Basch et al., 2005)

In one study, HIV-infected cells treated with alpha- and beta-momorcharin showed a nearly complete loss of viral antigen while healthy cells were largely unaffected. The name "Momordica" comes from Latin meaning "to bite," a reference to the jagged edges of the seed. The plant is aptly named, as all parts of the plant including the fruit taste bitter. Yet in Asia, where bitter flavors are enjoyed, bitter melon is popular.(Das, P., et al.2006). Bitter melon capsules and tinctures are becoming more widely available in the United States and are employed by natural health practitioners for diabetes, viruses, colds and flu, cancer and tumors, high cholesterol, and psoriasis. Concentrated fruit and seed extracts can be found in capsules and tablets as well as whole herb Bitter Gourd.(Russell, et al.,2009). one of the components of bitter melon extract may be effective in slowing the growth or spread of some types of cancer, particularly breast cancer. Cervical cancer patients (stage II or III) have shown some evidence of immune system response to bitter melon while undergoing radiotherapy in one poorly described study. Research in humans is limited, and more proven therapies are recommended at this time. Further study is needed before a recommendation can be made (Li, et al., 2009).

### Chemical constituents present in Bitter melon
Bitter melon contains an array of biologically active plant chemicals including triterpenes, proteins, and steroids. Alkaloids, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins, guanylate cyclase inhibitors, gypsogenin, hydroxytryptamines, karounidiols, lanosterol, lauric acid, linoleic acid, linolenic acid, momorcharasides,

momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordicosides, momordin, multiflorenol, myristic acid, nerolidol, oleanolic acid, oleic acid, oxalic acid, pentadecans, peptides, petroselinic acid, polypeptides, proteins, ribosome-inactivating proteins, rosmarinic acid, rubixanthin, spinasterol, steroidal glycosides, stigmasta-diols, stigmasterol, taraxerol, trehalose, trypsin inhibitors, uracil, vacine, v-insulin, verbascoside, vicine, zeatin, zeatin riboside, zeaxanthin, and zeinoxanthin are all found in bitter lemon.

## MATERIAL AND METHOD

### Collection and Authentication

The fresh whole plant of *Bitter melon* was purchased from local market in Jabalpur. then It was authenticated by Dr. A.B. Tiwari, Sr. Scientist, Department of Crop and Herbal Physiology, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (M.P).

### Preparation of extract

The whole plant was shade dried at room temperature and ground into fine powder. These fine powder was sieved to have a uniform size. After that take 25 gm of dried fine powder was extracted with different solvents viz, pet.ether, benzene, chloroform, acetone, ethanol and finally with water in soxhlet apparatus assembly. The extract was filtered using a whatman filter paper no. 4 and concentrated at 40°C; dried extract was refrigerated at 4 °C until use.

**Table 1: Extractive values of Rhizome of Bitter Melon.**

| S.No. | Name of Extract | Colour | Values in Percentage |
|---|---|---|---|
| 1. | Petroleum ether | Dark Brown | 3.52 |
| 2. | Benzene | Light Yellow | 2.80 |
| 3. | Chloroform | Light Brown | 0.30 |
| 4. | Acetone | Reddish Brown | 0.23 |
| 5. | Ethanol | Dark Red | 3.24 |
| 6. | Water | Brown | 12.31 |

## TLC STUDIES

TLC studies were performed by preparing slides of silica gel G. Sample of extract (10µl) were applied to each slide by micropipette. The slides were placed in suitable mobile phase and phytoconstituents was detected by specific detector reagent.

### $R_f$ value in TLC.

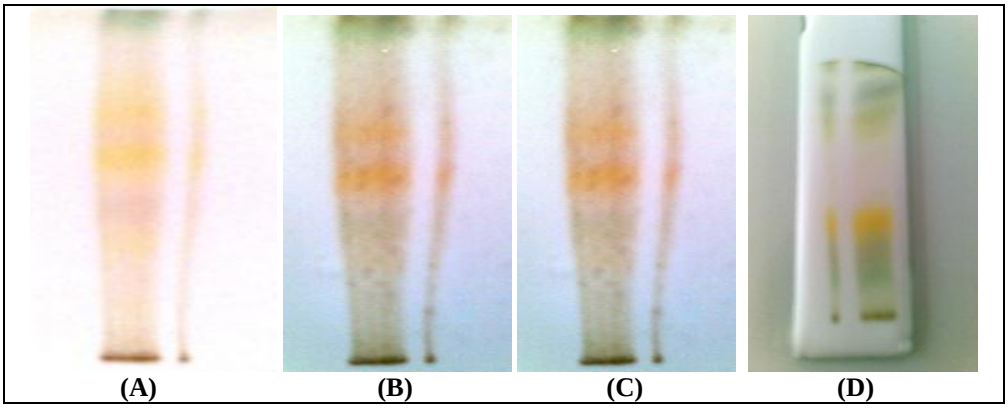
The migration distance of substances on thin layer chromatograms are generally fixed but some factors like thickness of the layer, chamber saturation, air humidity, separation efficacy of solvent mixtures etc. which are all difficult to reproduce can exert a marked influence on $R_f$ value (Mukherjee, 2002; Stahl, 1965; Heffman, 1966; Stahl,1973).

$$Rf = \frac{\text{Distance of centre of spot from starting point}}{\text{Distance of solvent front spot from starting point}}$$

**Table 2: TLC studies of ethanol extract of bitter melon.**

| S.No. | Mobile Phase | Detector | Observation | Possible Phytoconstituent |
|---|---|---|---|---|
| 1. | Ethyl acetate: Methanol: water (100:13.5:10) | Vanillin sulphuric acid | Red/yellow/blue-green | Bitter principle |
| | | Dragendorffs reagent | Orange , Red | Alkaloid |
| | | NP/PEG and UV | Yellow/green/orange | Flavonoid |
| 2. | Toluene:Ethyl acetate (93.:7) | VS reagent | Red/ yellow/Brown/Blue-green | Essential oil |
| | | NH3/KOH | Light Blue brown | Coumarin |

Photographic image of TLC profile



**(A) (B) (C) (D)**

**In Vitro Cytotoxicity Activity**

The anti-cancer properties of *Momordica charantia* are recently elucidated. Many researchers have found that treatment of *Momordica charantia* related products in a number of cancer cell lines induces cell cycle arrest and apoptosis without affecting normal cell growth. *Momordica charantia* derivative Alpha-eleostearic acid act by decreasing cell proliferation, G (2)-M block in the cell cycle, increase apoptosis on human breast cancer cell lines (Kobori M et.al 2008), and increase apoptosis in leukemia and colon cancer cell lines (Akihisa T et.al 2007). Oral administration of *Momordica charantia* extract (MCE) inhibits prostate cancer progression in transgenic adenocarcinoma of prostate mice by interfering cell cycle progression and proliferation as evidenced by reduced expression of proliferating cell nuclear antigen, and (ADP-ribose) polymerase (PARP cleavage). (Ru. P et. al 2011).

**MTT [(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrasodium bromide)] Assay.**

MTT [(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetra sodium bromide)] is a pale yellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria, and even freshly dead cells do not cleave significant amount of MTT. Thus the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. This assay was performed at Deshpande Laboratories, Bhopal using the standard operating procedures. Briefly the compounds was dissolved in DMSO and serially diluted with complete medium to get the concentrations a range of test concentration. DMSO concentration was kept < 0.1% in all the samples. Cell lines maintained in appropriate conditions was seeded in 96 well plates and treated with different concentrations of the test samples and incubated at 37 °C, 5% CO2 for 96 hours. MTT reagent was added to the well and incubated for 4 hours; the dark blue formazan product prepared by the cells was dissolved in DMSO under a safety cabinet and read at 550nm. Percentage inhibition was calculated and plotted with the concentrations used to calculate the IC50 values.

**Table 3: Cytotoxicity analysis of compound against Breast Adenocarcinoma.**

| Parameters | Results or specification |
|---|---|
| Assay | MTT |
| Time of incubation | 48h |
| Cell Line | MCF-7 |
| Organism | Homo sapiens (human) |
| Organ | Mammary gland; breast |
| Tissue | Epithelium |
| Disease | Adenocarcinoma |
| Derived from metastatic site | Pleural effusion |
| Receptors: | Estrogen receptor, expressed |
| DNA Profile (STR): | Amelogenin: X, CSF1PO: 10, D13S317: 11, D16S539: 11,12, D5S818: 11,12, D7S820: 8,9, THO1: 6, TPOX: 9,12, vWA: 14,15 |
| Cytogenetic Analysis: | Modal number = 82; range = 66 to 87.<br>The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4), markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic. |
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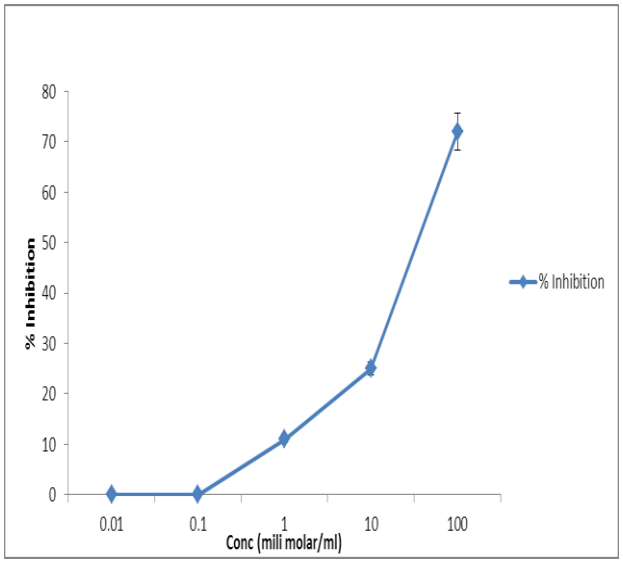
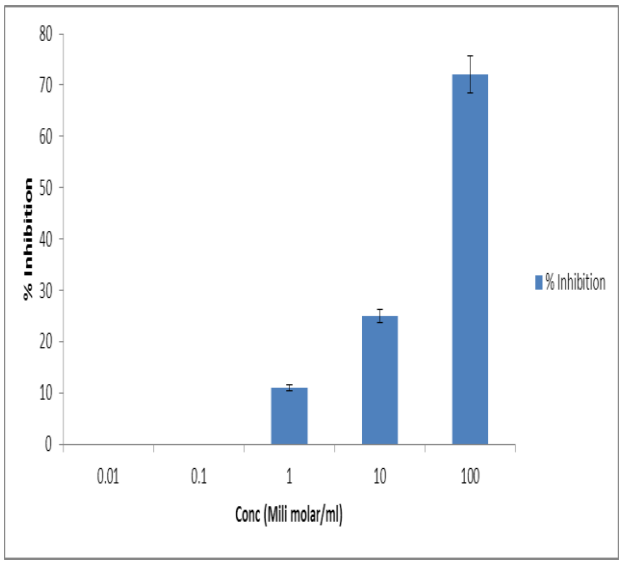
| Isoenzymes | AK-1, 1, ES-D, 1-2, G6PD, B, GLO-I, 1-2, PGM1, 1-2, PGM3, 1 |

**Table 4: Plant ext. shown the % Inhibition of cancer cell.**

| S.no. | concentration in milli molar/ml | % Inhibition |
|---|---|---|
| 1 | 0.01 | 0.00 |
| 2 | 0.1 | 0.00 |
| 3 | 1.0 | 11.0 |
| 4 | 10 | 25.0 |
| 5 | 100 | 72.0 |




**X axis** – concentration in milli molar
**Y axis**- % growth inhibition.




## SUMMARY AND CONCLUSION

The bitter melon plant (*Momordica charantia*) contains several seed lectins which are cytostatic or cytostatic to cells in culture, and which inhibit protein synthesis in vitro. We and others have isolated fractions with similar cytostatic and/or cytostatic activities from the bitter melon fruit. Vesely et al. and Claflin et al. reported that an aqueous extract of the ripe fruit contained both a guanylate cyclase enzyme inhibitor and a cytostatic factor which blocked rat splenic lymphocytes at the $G_2$ to M phase of the cell cycle. We have also found that similar exctracts prevent both concentration-A-stimulated thymidine incorporation into human peripheral blood lymphocyte DNA, and a subsequent induction of a specific cyclic AMP phosphodiesterase.

Natural products are a source for bioactive compounds and have potential for developing some novel therapeutic agents. Over the last decade there has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control. Among the known plant species, only a small percentage has been investigated for phytochemicals and pharmacological activities. Adverse side effects and high cost of modern medicine has intensified the search for other effective alternatives. Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy.

Bitter melon was subjected to extraction and phytochemical screening (Table1). TLC studies was also performed (Table 2) to ascertain the results of phytochemical screening. The phytochemical screening shows the presence of alkaloids, flavonoids and tannins. Thin Layer Chromatography revealed that ethanol gives better extraction of the phytochemicals than water since the ethanolic extract resolved into maximum number of bands as compare to aqueous extract. Herbal drugs are derived from heterogeneous sources leading to variations. This makes the standardization of herbal medicines all the more important as erroneous results can cause variations in pharmacological and phytochemical studies. Role may indicate that the growth and development of exposed organisms have been affected by test compounds. On the other hand, increasing values are result of the induction of cell division, and may be characterized as detrimental event to cells by leading to both uncontrolled proliferation and tumour formation.

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